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THE MAGNESIUM REQUIREMENT OF FUNGI

BY

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I. INTRODUCTION

The question of the mineral nutrition of the fungi has attracted the attention of many investigators, yet the subject is still in a very unsatisfactory state. The number of different forms with which investigators have experimented is relatively small and the cultural conditions and methods have been quite similar in all cases. In general, we know which elements are essential for normal growth and reproduction of fungi. Further experimentation is necessary to extend our present knowledge to a greater number of forms and to study the problems under more varied cultural conditions. Certain factors render work of this kind very difficult.

It has been impossible thus far to secure cultural flasks, the constituents of which will not dissolve to some degree in the nutrient solution, either at the time of sterilization or later, during the growth of the fungi, when the medium may develop an acid reaction. Such a property of the cultural flasks permits of the introduction of impurities into the nutrient medium, thereby causing a source of error.

It is very difficult to remove all traces of non-essential substances and impurities from the chemicals, which are to be used in the making of nutrient solutions. The nature of the organisms to be used in the experiments is also an important consideration, especially since their

sensitiveness to extremely small quantities of essential elements may often vitiate the results.

II. LITERATURE

a) *Magnesium as an essential element*

PASTEUR (1864) was perhaps the first investigator to grow fungi in artificial media. In an experiment which bears the date, April 9, 1862, he included magnesium phosphat among the constituents of the nutrient solution which he used for growing *Mycoderma aceti*. The amount of magnesium phosphat used was one-tenth of a gram to a liter of solution. Although he gave no accounts of any experiments to show that magnesium is an essential element for the growth of Mycoderma, it is quite obvious from his work that such was his belief. In an earlier paper (1860), he cited an analysis of yeast, which showed magnesium oxid to be one of the ash constituents. Evidently from this fact, he assumed that magnesium was a factor in the metabolism of yeast, and, therefore, he included a magnesium compound in nutrient media for *Mycoderma aceti*.

The classical work of RAULIN (1869) was the first definite evidence furnished as to the importance of magnesium in a nutrient solution. He worked with very complex solutions, which contained varying amounts of sugar, tartaric acid, ammonium nitrat, zinc carbonat, citrat of iron, silicat of potassium, ammonium phosphat, potassium carbonat, mag-

nesium carbonat and ammonium sulphat, which he used as a standard nutrient solution. To test the importance of an element, he introduced the method still used at the present time. The element to be tested is omitted from the nutrient solution and then the growth, which is produced on such a solution, is compared with the amount and character of that produced on a standard nutrient solution during the same interval of time, and under the same cultural conditions. RAULIN (1863) grew *Aspergillus niger* in his standard nutrient solution and in the same solution minus the magnesium constituent. In the former case the growth was 11.6 times greater than in the latter. In other similar experiments, he obtained growths as high as 91 times as great on the standard nutrient solution, as on the solution to which no magnesium had been added. Such a difference in the amount of growth in the two solutions RAULIN states, "proves evidently the efficiency of the magnesium". He also stated that "these experiments leave no doubt as to the physiological usefulness of magnesium". It may also be noted that RAULIN concluded that the mineral oxides (magnesium included) of artificial media cannot replace each other, to each of which is assigned a special rôle. It is evident, therefore, that he considered magnesium as an essential and a non-replaceable element.

ADOLF MAYER (1869) also published a paper in which he showed that magnesium was necessary for the complete develop-

ment of beer yeast. He grew his yeast in 35 cc. flasks to which had been added 20 cc. of a nutrient solution containing:

0.1 g. mono-basic potassium phosphat
0.05 g. magnesium sulphat (+7 H_2O) crystals
0.001 g. tri-basic calcium phosphat
20 cc. of a 15% sugar solution
0.15 g. ammonium nitrat.

After sterilizing his solutions by heating in a sand bath, he inoculated them with minimal amounts of yeast, and then used the rate and degree of fermentation as criteria for the suitableness of the medium. In nutrient solutions, to which no magnesium sulphat was added, the yeast produced only a very feeble fermentation, hence MAYER states "the presence of a magnesium salt must be seen as truly necessary to the normal progress of fermentation". In spite of the fact that he obtained a certain degree of fermentation in cultures to which no magnesium sulphat was added, MAYER concludes that the yeast, Saccharomyces cerevisiae, requires a magnesium salt for its complete nutrition.

MAYER (1886) stated in his "Lehrbuch der Gährungschemie" that Mg - salts cannot be replaced by calcium salts in the nutrient solution of yeast. It apparently makes no difference whether the magnesium is supplied to the yeast as sulphat or as ammonium magnesium phosphat. MAYER states that "the beer yeast cannot continually dispense with magnesium, even if it does succeed in supporting a fermentation of con-

siderable intensity by the greatest removal possible of magnesium".

NÄGELI (1879) comes to a conclusion quite different from those of previous workers. He concludes that magnesium can be replaced by either calcium, barium, or strontium. He states that his experiments give only general certainty as to the replaceability of the four named elements. NÄGELI is sure that either Mg, Ca, Ba or Sr can render growth possible, and that without one of these growth is impossible. His experiments leave him in doubt as to whether these four elements are equally efficient in nourishing the fungus, or whether the fungus is better nourished by one alone, by a combination of two, or by a combination of more than two of these elements. NÄGELI recognized the fact that only small amounts of minerals are necessary in order to nourish the fungi; thus in experiments,which determine this requirement, great care must be exercised to use pure chemicals and clean cultural flasks. Evidently his chemicals were not pure but contained traces of magnesium, which altered his results and led him to draw wrong conclusions. The majority of NÄGELI'S experiments were performed with beer yeast.

O. LOEW (1879), working with certain molds, came to conclusions similar to those of NÄGELI, namely that Mg, Ba, Ca and Sr may replace each other in a nutrient solution. Penicillium was grown on a standard nutrient solution con-

taining a half liter of a 3% solution of ammonium acetate, to which was added 1% di-potassium phosphat, 0.04% ammonia dithionat, 0.016% magnesium chlorid and 0.006% calcium chlorid. The solution was acidified with 1% acetic acid in order to prevent bacterial growth. Growth on (a), the standard nutrient solution containing both Mg and Ca was compared with that on nutrient solutions containing: (b) magnesium only; (c) calcium only; (d) neither magnesium nor calcium; (e) magnesium and barium but no calcium; (f) magnesium and strontium but no calcium; (g) barium only; (h) strontium only. Where only calcium and only barium were present the liquid assumed a red color, and next to those of the standard solution, these cultures produced the largest number of spores. On the remaining cultures, the sporulation was very weak or entirely lacking. The spores were, at all times, of a reddish color. After seven weeks the crops on the various cultures were collected and dried yielding the following amounts of dry weights.

a.	Mg,	Ca	0.498 g.
b.	Mg,	__	0.153
c.	__,	Ca	0.491
d.	__,	__	0.026
e.	Mg,	Ba	0.201
f.	Mg,	Sr	0.190
g.	__,	Ba	0.216
h.	__,	Sr	0.103

LOEW states that it results that by the absence of alkali earths in (d) only a minimal amount of mold develops,

and that each of the four elements used, can by the molds replace each other to a certain degree. He concludes that the minimal development in the solution to which neither calcium nor magnesium was added, is brought about at the expense of the mineral matter contained in the spores at the time of sowing.

The above report of the experiments by LOEW was incorporated in the paper presented by NÄGELI to the Munich Academy in (1879). Evidently LOEW worked under the supervision of NÄGELI , using the same technique and undoubtedly deriving his chemicals from the same source, therefore it is not surprising that LOEW should have encountered the same difficulties and should have drawn the same erroneous conclusions as did NÄGELI.

WINOGRADSKY (1884) proved that magnesium was necessary for the growth of Mycoderma vini. He grew this fungus in Erlenmeyer flasks and used a nutrient solution which contained glycerin, alcohol, asparagin, malic acid, ammonium phosphat, magnesium sulphat and calcium acetat. Growth on this solution was compared with that on solutions in which substitutions had been made in the alkalin earth constituents. In the first series the culture liquid contained $MgSO_4$; in the second series an equivalent amount of $CaSO_4$; in the third an equivalent amount of $SrSO_4$; in the fourth no alkalin earths but an equivalent amount of potassium sulphat. In the

flasks containing the $MgSO_4$, the fungus grew very well, but in the other flasks hardly any development took place. These results contradict the observations of NÄGELI, who found that anyone of the alkalin earths is sufficient for the nutrition of the fungi studied by him. WINOGRADSKY showed that magnesium cannot be replaced by either calcium or strontium and that as far as the nutrition of Mycoderma vini is concerned, magnesium is an absolutely non-replaceable element.

SESTINI (1888) showed that beryllium can replace magnesium in the nutrition of legumes and grasses. He grew his plants in sand cultures and watered them in one series with a magnesium-containing nutrient solution, and in a second series with a nutrient solution, whose magnesium constituent had been replaced by a beryllium compound. The legumes grew in both series but developed only a few flowers in either case and produced no mature fruits, although pods developed. The grasses developed mature fruits in both series. The individuals cultivated with magnesium developed a larger number of fruits and each of the plants thus grown possessed a higher weight than those grown in the beryllium series. The ash analysis of the grain producing plants, grown in the beryllium nutrient solution, showed that the plants had actually taken up beryllium, as this element formed about two percent of the ash. Traces of magnesium were also demonstrated to be present in the ash, but SESTINI concludes that this is due to the magnesium content of the

seeds from which the plants were grown.

SESTINI (1891) extended his work to the Zea mais, employing similar methods to those used in his previous work. Sand cultures were employed and eight liters of nutrient solution, containing 1.44 grams $MgSO_4$ were used to water the plants in the first series. In the second series, an equal volume of nutrient solution was used, containing 1.6 gr. $BeSO_4$, being the equivalent amount of beryllium to the magnesium contained in the first nutrient. The plants reached a height of 90 - 95 cm., and bore mature ears, yet the plants in the beryllium series were less vigorous than those in the magnesium series, as the following data show:-

	Mg plants	Be plants
Number of grains	322	283
Weight of grains	15.20 g.	12.31 g.
Weight of one grain	0.472 g.	0.435 g.

The ash of the beryllium plants contained 2% Be-oxid. The seeds obtained from the beryllium plants were planted as before and watered with the Be-nutrient solution. Of twenty seeds planted, only seven germinated and but three of these plants bore fruits. From these fruits, fourteen corn grains were obtained and their total weight was only 0.37 gr., a single grain weighing but 0.026 g. These results show that the embryos produced in the beryllium series were weak and only in a small degree capable of germination. SESTINI con-

cludes that a part of the functions of magnesium can indeed be assumed by beryllium; beryllium can replace magnesium during the vegetative period of plants, but flowers and viable seeds cannot be formed abundantly and when seeds are set they are very weak.

O. LOEW (1892) studied the effect of the magnesium content of nutrient solutions, upon the fermenting capacity of putrefying bacteria. He made up a peptone solution and divided it into five parts. To the first part no salts were added, to the second part, 1% of KNO_3 and to the third, fourth, and fifth parts, respectively 1% of $NaNO_3$, $Ca(NO_3)_2$ and $Mg(NO_3)2$. All five cultures were infected with putrefying bacteria and placed at room temperature. After ten days, the intensity of the ammonia formation was determined and found to be most intense in the control and the magnesium containing cultures. Ammonification was only half as strong in the Na and K cultures, and only one-third as strong in the calcium culture. LOEW also showed that yeast is not injured in the least, if warmed for hours at 25 - 30°C with a 1% solution of $Mg(NO_3)_2$. Magnesium salts in the absence of calcium salts are, therefore, not injurious to bacteria and yeast. These results show a marked contrast to the toxicity of magnesium for green plants.

BENECKE (1894) refuted the work of NÄGELI and LOEW and also obtained results contradictory to those of SESTINI. In

the various nutrient solutions which he used, BENECKE found the presence of magnesium to be absolutely necessary for the growth of _Aspergillus niger_ and _Penicillium glaucum_; calcium was of no value, while barium and strontium, especially the former, are directly injurious. As cultural flasks, he used those of ordinary Thüringer or Jena glass, taking particular pains to clean the flasks so that constituent substances would not enter the nutrient solution during the experiment. The chemicals were purified by repeated crystallization, the glycerin was redistilled, and the sugar was purified by many precipitations with alcohol. The water was distilled with particular care in a tinned apparatus. Glycerin in particular, but also cane sugar, glucose, acetic acid, tartaric acid and asparagin were used as sources of carbon. Nitrogen was supplied either in the form of ammonium compounds or as nitrates. Salts were added as acid potassium phosphat and the alkalin earths as sulphates or nitrates. BENECKE used cultural solutions containing from 25 - 100 cc. of solution. Aspergillus was grown at a temperature of from 20 - 30°C while Penicillium was kept for the most part at room temperature.

Although without an addition of magnesium, germination of the spores occurred, however, the growth which followed was always very weak and was evidently brought about by impurities. BENECKE states, "that a minimal germination without the

addition of magnesium is easily conceivable as being at the cost of the exceedingly slight traces of magnesium which are in the spores when sown". Zinc, cadmium and beryllium, which stand close to magnesium in the periodic system, were found to be unsuitable for replacing the latter in nutrient solutions. All three of these elements were poisonous. Beryllium was not found to be a strong poison for fungi; only when this element is concentrated in considerable amounts, does a poisonous affect appear. Zinc and cadmium especially the latter, were found to be very poisonous.

BENECKE found that in a normal weakly acid glycerin nutrient solution of 100 cc. content, an addition of 0.001% $MgSO_4 + 7\ H_2O$ is sufficient for the formation of a strong fungus mat with conidia; by an addition of 1/2 mg. or 0.0005% $MgSO_4 + 7\ H_2O$ the conidia remain brown and evidently fail to ripen, while in the presence of a still smaller magnesium content, spore formation is completely suppressed and the growth produced appears only as a white mycelial mat.

BENECKE grew Triticum in water cultures that contained a beryllium compound in place of the magnesium constituent. He was unable to corroborate SESTINI'S results, but found instead that the beryllium exerted a poisonous influence. During the course of six weeks the beryllium plants could not keep pace with normally nourished plants, but remained quite perceptibly behind those in the magnesium free cultures.

MOLISCH (1894) came to identically the same conclusions as BENECKE. He used extreme care in the preparation of his nutrient solutions. The chemicals were bought in the purest condition possible and were recrystallized two to four times, or, as in the case of ammonium chlorid, were sublimed. The organic nutrients as glycerin and alcohol were distilled. The cultures were made in Erlenmeyer flasks plugged with cotton. MOLISCH used Penicillium sp. and Aspergillus niger in all of his experiments. When ammonium acetat was used as a source of carbon and no magnesium was added to the nutrient solution, no traces of growth of either of the two fungi occurred. In the experiments, where sugar was used as a carbon supply, the results were not as marked for in the nutrient solution to which no magnesium was added some mycelial growth took place, but no spores, however, were formed. MOLISCH concludes that the partial growth of the fungus is no doubt due to traces of magnesium which could not be separated from the sugar in spite of the most careful purification. From the results of his experiments, MOLISCH comes to the conclusion that magnesium is an integral constituent of the mineral nutrition of the lower fungi and that it is impossible to replace this element either by the closely related elements Ca, Ba or Sr, or by those of the zinc group, Zn, Be and Cd. $CdSO_4$ even in such dilute solutions as 0.02%, 0.01% and 0.002% acts as a

poison on Aspergillus, Penicillium, and various bacteria. $CdCl_2$ was found to exert a similar toxic effect.

BENECKE (1895) further extended his studies on the mineral requirements of the fungi and concluded that the replacement of magnesium by the alkalin earths or other allied bases, is strictly impossible. BENECKE used Aspergillus niger and Penicillium sp. as the objects of his investigation. A series of six flasks, each containing 100 cc. of nutrient solution were inoculated with Penicillium. The first flask contained no addition of alkalin earths, the second 0.02% $Ca(NO_3)_2$, the third 0.04% $Ba(NO_3)_2$, the fourth 0.03% $Sr(NO_3)_2$, the fifth 0.02% $MgSO_4.7\ H_2O$, the sixth 0.02% $MgSO_4$ + 0.02% $Cu(NO_3)_2$. The spores germinated in all the flasks, while in the last two flasks, those containing magnesium, the fungus reached the spore bearing stage. In other cultures, barium and strontium were again shown to be poisonous.

BENECKE conducted a series of ten experiments with Aspergillus niger, in which he varied the $MgSO_4(+ 7\ H_2O)$ content from 5% to 0.0%. After 27 days the flasks containing 0.02% $MgSO_4$ showed the greatest development of fungus, With the Mg - content below 0.02% the fungus weight and the spore production decreased until at a concentration of 0.00008%, there were scarcely any spores produced. The development on the cultures, to which no magnesium was added, was too

small to be weighed. Sporulation, in the flask containing 5% $MgSO_4$, was very heavy but the mycelial development was only about one-third that on the 0.02% $MgSO_4$ cultures. The cultures containing 1.25% $MgSO_4$ and 0.0049% $MgSO_4$ respectively, showed exactly the same amount of mycelial growth. BENECKE states that "magnesium is a metal, which no plant can do without for its complete development, and further, magnesium has a bearing upon the life of every cell".

WEHMER (1895) reviewed the literature dealing with the mineral requirement of fungi and performed some experiments of his own. He gives no detailed accounts of his experiments but simply states that his results do not entirely harmonize with those of other workers. The explanation for this, he thinks is perhaps due to the fact that "for each organism and also for every species of fungus, there is an optimum of conditions relating to the life processes; not only such conditions as temperature and the like, but also that of the concentration and composition of the substratum".

BENECKE (1896) further worked on the relation of magnesium to the growth of _Aspergillus niger_, _Penicillium_ sp., _Mucor stolonifer_, and yeast. He was extremely careful in the preparation of his nutrient solutions, and furthermore, made a preliminary study of the chemical composition of his culture flasks and determined to what degree his nutrient solutions were changed by the solubility of the glass.

BENECKE showed that considerable difference could be recognized in cultures of Aspergillus niger which contained no added $MgSO_4$(+ 7 H_2O) and in cultures to which 1/400 mg. of this salt was added to 25 cc. of nutrient solution. He also grew Aspergillus niger in solutions which contained from 0.01 to 0.0000025 grams of crystallized $MgSO_4$ to each 25 cc. of solution. The weight of the fungous crop as well as the "economic coefficient" fluctuates with the Mg - content. In the magnesium free cultures a few small masses of submerged mycelium developed, but grew no more and never sporulated even if allowed to stand for a long time. By acidifying his nutrient solutions with organic acids, BENECKE proved that the more strongly the solution was acidified, the more Mg must be present in order to turn the solution into an available nutrient solution. The presence of acids tended to suppress the growth of the fungus.

GÜNTHER (1897) concluded that a magnesium salt was necessary for good nutrition of Mucor corymbifer, Rhizopus nigricans, and Botrytis cinerea. His technique was identical with that used by MOLISCH. He showed that 0.005 mg. of $MgSO_4$(+ 7 H_2O) was a minimal addition to 25 cc. of nutrient solution to induce Rhizopus nigricans to grow at all. GÜNTHER also proved that magnesium salts cannot be replaced by those of Ca, Ba, Sr, Be, Zn or Cd. The toxicity of these salts to Aspergillus nigricans was determined and the results show that the Cd - salt is the most injurious, while the

calcium salt is the least poisonous. The following concentrations were sufficient to inhibit growth:- 0.0001% $CdCl_2$-, 0.01% $ZuSO_4$, 1.% $Ba(NO_3)_2$, 1.5% $Sr(NO_3)_2$, 0.2% $BeCl_2$, 4% $Ca(NO_3)_2$. It is seen that beryllium is less poisonous than either Zn or Cd. Beryllium chlorid may be tolerated up to 0.2%. Concentrations, however, somewhat lower than this brought about peculiarities of growth.

GÜNTHER also determined in a few cases the maximum concentration of $MgSO_4$ which Rhizopus nigricans could tolerate. Above 15% of $MgSO_4$ no more growth occurred. Magnesium nitrat was found to be more injurious. If the concentration of $Mg(NO_3)_2$ was greater than 5% development was checked.

COUPIN (1903) worked on the nutrition of Sterigmatocystis nigra. He used RAULIN'S nutrient solution, in one-fifth the original quantity of liquid, and repeated RAULIN'S work in the light of modern technique. Grown on a complete nutrient solution, the fungus produced a dry weight of 4.590 grams, while grown on a nutrient solution minus the magnesium constituent, the fungus obtained a dry weight of but 0.020 grams. The ratio of the weight of the fungus in complete media, to the weight of the fungus in media deprived of $MgCO_3$, COUPIN finds to be 229.4.

KOSSOWICZ (1903) showed that the pigment formation in certain species of Saccharomyces depends upon the presence and amount of $MgSO_4$. Under most favorable conditions, the

pigment formation begins at a $MgSO_4$ content of 0.04% and appears sooner and with greater intensity, the higher the content of the $MgSO_4$. From 4% $MgSO_4$ up to a complete saturation of the nutrient solution with this salt, the coloration is most evident. By increasing the amount of $MgSO_4$, various color shades were noticeable, from light blood red and rose red to a dark brickish red.

COUPIN and FRIEDEL (1904) state that Sterigmatocystis versicolor requires magnesium for one of its nutrients, as its absence caused a considerable decrease in the dry weight of the fungus. These investigators call attention to the fact that, in RAULIN'S solutions minus the magnesium component, the spores of the fungus were of a rose grey color, instead of the normal green color. They obtained, however, a growth of Sterigmatocystis even to the spore bearing stage, without an addition of magnesium.

BENECKE (1905) in a review of the literature of the magnesium requirement of fungi, cited the work of COUPIN and FRIEDEL and stated that, in the light of their experiments, it is still an open question whether or not magnesium can be replaced, at least in part, by other alkaline earths. He thought that a replacement of magnesium by calcium might be possible.

FROUIN (1912) studied the development of tubercular bacteria and Aspergillus niger in relation to the salts of

the rare earths. He used the sulphates of cerium, lanthanium, neodymium, praesodymium, thorium, samarium and yttrium. No matter in what quantity the salts of these elements were substituted for magnesium, negative results were always obtained. Therefore, the conclusion was reached that the salts of the rare earths were unsuitable for replacing magnesium salts.

BUROMSKY (1912) studied the influence of magnesium on the development of _Aspergillus niger_. He was very careful in the purification of his chemicals and in the distillation of the water which he used. Culture flasks of 1330 cc. capacity were employed, to each of which 220 cc. of nutrient solution were added. This amount of solution gave a depth of one centimeter in the flask so that a broad surface was exposed to a large quantity of air above it, a factor which BUROMSKY states to be very necessary for continued cultures. Flasks were plugged with cotton and sterilized twice in a steam sterilizer, each time for one-half hour, with an interval of twenty-four hours. All his cultures were placed in a dark room at 29 - 30° C. After the experiments were ended, the mycelium of the fungus was thoroughly washed and dried to constant weight at 60°C. The nutrient solution, which BUROMSKY used, contained:-

0.50 g. KH_2PO_4
0.25 g. $MgSO_4$
1.0 g. $(NH_4)_2SO_4$ or NH_4NO_3
A trace of $FeSO_4$
5.0 g. cane sugar
100 cc. H_2O

In cultures containing no addition of magnesium, BUROMSKY found that the spores germinated but further development was very weak. The mycelium grew in small tufts, which were usually submerged, never producing a mat on the surface, as in the case of the normal cultures. BUROMSKY used concentrations of $MgSO_4$ of 0.125, 0.25 and .50% and in all these concentrations the fungus developed equally well; however, 0.5% was found to be the highest concentration which would permit normal development, as raising the concentration above this point caused a marked decrease in sporulation. Fewer spores were formed with the 0.5% concentration than with the 0.25%.

In cultures to which neither $CaSO_4$ nor $MgSO_4$ were added, BUROMSKY found an average dry weight of fungus equal to 0.196 grams; those to which 0.25% $CaSO_4$ was added, an average of 0.099 grams; 0.25% $MgSO_4$, an average of 2.144 grams; while those to which both 0.25% $MgSO_4$ and 0.25% $CaSO_4$ were added gave an average of 2.40 grams. Thus it was found that $CaSO_4$ in the absence of $MgSO_4$ caused a depressant effect, while in the presence of $MgSO_4$ it caused a stimulatory effect. By increasing the percentage of $MgSO_4$ in the nutrient solution, BUROMSKY found that the weight of fungus produced was increased. The respiratory coefficient decreased as well as the economic coefficient. The respiratory coefficient in Mg-free cultures averaged 6.4, while in Mg-containing cultures, it fell to 3.2.

The economic coefficient of 10 for Mg-free cultures dropped to 3.35 in cultures with $MgSO_4$. BUROMSKY concludes that magnesium is not only an essential and non-replaceable element for the growth of _Aspergillus niger_, but that it produces a stimulatory effect on the growth of the mycelium.

JAVILLIER (1913) attempted the substitution of magnesium in culture media by beryllium in the growth of _Aspergillus niger_. He used a RAULIN nutrient solution and grew the fungus for four days at 34°C, after which he determined the dry weights. In cultures to which either equivalent weights or equivalent molecular quantities of $BeSO_4$ were substituted for $MgSO_4$, no growth or even germination of spores occurred. JAVILLIER concludes that magnesium is an absolutely essential element for the growth of Aspergillus and furthermore that it can not be replaced by beryllium. He states that experiments conducted independently of his, by SAUTON, furnished analogous results.

DOX (1914) reviews the more recent literature on the mineral nutrition of fungi, but he gives no account of any original experiments showing the importance of magnesium.

b) _Magnesium in relation to the nutrition of bacteria._

The magnesium requirement of bacteria has been studied by many investigators but uniform results have not been obtained and hence this question is still in a very unsettled condition.

GESSARD (1892) studied the fluorescing power of bacteria and showed that the phosphorus content rather than the magnesium content was the essential factor.

MOLISCH (1894) found that Mg-free cultures remained sterile when inoculated with putrefying bacteria.

THUMM (1895) worked with various races of _Bacillus pyocyaneus_ and _Bacillus viridans_ and found that $MgSO_4$ was necessary for pigment formation. A scattered growth of bacteria with no pigment formation occurred in cultures to which no magnesium was added. THUMM concludes that the colorless growth is due to traces of Mg which are not removed from the culture media. He also proved that for pigment formation magnesium can never be replaced by calcium. An addition of 0.00001% $MgSO_4$ was sufficient to permit pigment formation in _Bac. viridans_. However, he found that most forms require from .01% - 0.001% $MgSO_4$ for this function.

NOESSKE (1900) showed _Bac. pyocyaneus_ and _Bac. prodigiosus_ to be very sensitive to only small amounts of Mg-salts. By increasing the amount of magnesium, pigment formation definitely increased, while growth was still more vigorous. For _Bac. pyocyaneus_, magnesium can not be replaced by Be, Cd or Zn.

KUNTZE (1900), working with _Bacillus prodigiosus_, obtained growth but no pigment formation, when no magnesium was added to his media. An addition of 0.001% $MgSO_4$ was sufficient to cause the production of pigment. When Mg was present,

growth was always stronger than when magnesium was absent.

JORDAN (1899), after studying six different species of fluorescent bacteria, concludes that the sulphat, rather than the magnesium of $MgSO_4$ is the important factor in pigment formation.

McKENNEY (1902) determined the conditions necessary for light production by _Bac. phosphorescens._ He showed that .1% $MgCl_2$ was insufficient and that 0.25% $MgCl_2$ was required to cause growth, while 1% $MgCl_2$ was necessary for light production; 5% $MgCl_2$ inhibited light production. He considers that magnesium may be replaced by sodium.

SAMKOW (1903) proved that magnesium is necessary for pigment formation by _Bacillus prodigiosus_. He extracted the pigment of this bacillus and analyzed it, finding no magnesium present. SAMKOW concludes that "the appearance of the red pigment of _Bacillus prodigiosus_ is affected by the presence of magnesium, but this element forms no constituent of the pigment."

The relation between the magnesium content of the medium and the pigment formation of _Bact. syntanthum_ was studied by KOSSOWICZ (1903) who observed that the appearance and the intensity of the reddish brown pigment formed, was influenced by the $MgSO_4$ content.

BENECKE (1907) in an exhaustive study of the mineral nutrition of bacteria, concludes that for _Bac. fluorescens_

and Bac. pyocyaneus magnesium is not only a special pigment forming element but its presence is also indispensable for growth and cannot be replaced by calcium. BENECKE performed many experiments to show that sufficient amounts of magnesium for growth can be dissolved from the glass flasks. He states that this is perhaps the reason why he obtained a growth of Aspergillus and Penicillium in cultures to which no magnesium had been added. In conclusion, he adds that "a fungus which can grow without the presence of magnesium, has not yet been found".

c) The function of magnesium.

As to the specific rôle of magnesium in the metabolism of the plant very little is known. Most investigators, who consider that magnesium is essential for growth, make no attempt to define its particular function.

LIEBIG (1846) long ago concluded that the alkalies and the alkalin earths were necessary to neutralize the organic acids formed in the plant. He considered that these elements were capable of replacing one another. RAULIN (1869) stated that experimentation did not favor LIEBIG'S hypothesis. He evidently believed that magnesium was a constituent of the protoplasm of cells.

PFEFFER (1871) demonstrated the presence of magnesium in protein grains. SCHMIEDEBERG (1877) found the same to be true for the protein crystals of Brazil-nuts. GRÜBLER (1881)

proved that crystallizable protein of the gourd contained magnesium in considerable quantities.

NÄGELI (1879) stated that the alkalin earths were found as deposits in the plasma and in the cell membrane. He conceived that the salt molecule was in some way fixed on the surface of the albuminat and on the surface of the cellulose micellae. He calls attention to the fact that the alkalin earths, with which he worked, were with difficulty soluble or were insoluble, and, therefore, easily accessible due to the attraction of the organic substances within the cell.

LOEW (1892) found the oxidative capacity of fungi to be less intense under the influence of magnesium salts than under that of other salts.

BENECKE (1895) remarked that magnesium was of value in the nutrition of the fungi because of its basic properties. Magnesium salts readily dissociate and, as **PFEFFER** and **SCHMIEDEBERG** have shown, magnesium has the capacity to link itself to some organic acid radicle within the cell.

SAMKOW (1903) showed that magnesium acts as a catalytic agent in the formation of pigment by bacteria. Many other workers, as pointed out above, have demonstrated the importance of magnesium in relation to pigment formation but none have offered any explanation as to the function of magnesium in this process. **JORDAN** considered the pigment to be a by-product in the metabolism of the bacteria, hence from this it might

be concluded that only in so far as magnesium is necessary for life does it influence the formation of the pigment.

Analyses of plant ash (ZELLNER, 1907) have shown magnesium as a constituent part of all plants. A greater content of magnesium is usually found to occur in spores and seeds, than in vegetative tissue.

ASO (1900) analyzed the spores of Aspergillus oryzae and found 4.364% of magnesium oxid present. He concludes that magnesium plays "an important rôle in the assimulation of phospheric acid. Wherever proteins are formed with accompanying development, and phosphates are present, there magnesia always is found." ASO states that phosphoric acid can be more easily assimilated from magnesium phosphat than from any other phosphat due to the ease of dissociation of magnesium salts.

For green plants, WILLSTÄTTER and his co-workers have shown that magnesium is a constituent part of chlorophyll.

As pointed out above SESTINI was able to partially replace magnesium by beryllium in the nutrition of legumes, corn and grasses. BENECKE stated that magnesium owed its position in the nutrition of plants to its basic properties. Since very little work has been reported on the replacement of magnesium by beryllium in the growth of fungi, and, since beryllium salts like those of magnesium are easily dissociated, it seemed desirable to study the problem of the substitution of magnesium by beryllium in the nutrition of

Aspergillus niger, a plant widely used in similar experiments.

III. REAGENTS AND METHODS

The chemicals used for this work were of the best grade obtainable under the present conditions of supply. Merck's "Blue Label" analyzed chemicals were used wherever possible, Baker's analyzed chemicals were used in some cases, Kahlbaum's in others. Sucrose was obtained in the form of rock candy crystals. Further purification of the chemicals was not attempted, but extreme care was exercised in handling them in order to prevent the introduction of foreign materials and impurities. The water used was twice distilled from Jena glass. Potassium bichromat cleaning solution to the amount of five cubic centimeters per liter was added during the first distillation, while five cubic centimeters of conc. H_2SO_4 were added during the second distillation.

All solutions used were made up in molecular strengths, excepting the ferric chloride which was made up as a one-hundredth molecular solution. The Sodium sulphat had to be made up as a one-half molecular solution, because of the insolubility of this salt.

Jena glass Ehrlenmeyer flasks of 100 cc. capacity were used in all cases as cultural flasks. The flasks were

first treated with potassium bi-chromat cleaning solution for at least twelve hours, rinsed in tap water and in distilled water and then placed over a jet of live steam from distilled water boiled in a Jena glass flask. After steaming the flasks for five minutes they were drained and dried in a hot air oven, and then plugged with cotton till ready for use. All glassware used received the same treatment.

As a standard nutrient solution, one was used which is employed in the cultural work at the Advanced Plant Physiology work of this laboratory. This solution consists of: 125 cc. M. NH_4NO_3, 50 cc. M. KH_2PO_4, 20 cc. M. $MgSO_4$, 5 cc. M/100 $FeCl_3$, 50 grams (dry wt.) sucrose made up to 1 liter with redistilled water. The solutions, from which nutrient solutions were made, were measured by placing them in burettes, while the nutrient solutions were always measured in volumetric flasks. No nutrient solutions were made up until they were desired for immediate use.

Fifty cc. of nutrient solution were used for each culture. The solution was sterilized by placing in an autoclave at five pounds pressure for one-half hour. Higher pressures were avoided in sterilizing in order that the nutrient solution would be altered as slightly as possible.

Spores of *Aspergillus niger* were used in making all

inoculations. The spores were obtained by growing the fungus on potato agar slants. The spores used for inoculation were always from fresh cultures, in order that they might be readily viable. All the plants were from one strain and not from a mixed culture.

Inoculation was accomplished by means of the water blank method. Ten cc. of sterile redistilled water received a heavy addition of spores, precaution being taken not to introduce fragments of mycelium along with the spores. By means of a sterile pipette, three drops of this spore-containing water was added to each flask. This method of inoculation gave very satisfactory results, as the spores seemed to be evenly distributed in all the cultures. In all the cultures which were made, not one showed any signs of contamination.

Most of the cultures were either placed in a dark room or in a cabinet beneath the laboratory table. The temperature in these two places ranged from 20 - 23°C; one or two cultures from each series of experiments were placed in an incubator at 30°C in order to determine whether the fungus might not produce a better growth at the higher temperature. The early stages of growth were always more rapid, however, when the cultures were grown at 30°C, the end result was always about equal to those grown at room temperature.

All cultures were allowed to grow from ten days to three weeks, during which time observations were made on the manner of growth. At the end of each experiment, the mycelium of each flask was collected in a Gooch crucible, washed and dried to constant weight at 100°C.

IV. DESCRIPTION OF EXPERIMENTS

Experiment I.

As a preliminary experiment, the replacement of Mg by sodium was attempted. The nutrient solution used was made up as follows:

1000 cc. nutrient sol.

125 cc. NH_4NO_3	5 cc. (M/100) $FeCl_3$
50 cc. KH_2PO_4	50 gr. sucrose
35 cc. (M/2) Na_2SO_4	

One series of ten flasks was filled with this solution; a second series of ten flasks contained the standard nutrient solution, while a third series of five flasks contained a solution made by mixing equal parts of those used in series one and two. The cultures were grown at 22°C for a period of 20 days. The cultures containing the Mg, and those containing the Mg + Na were very similar in appearance. Both growth and sporulation were very heavy. The cultures containing Na but no Mg produced only scattered tufts of mycelium which never produced spores. Most of the mycelium

grew submerged. The dry weights were as follows:

Mg Series		Na Series		Mg + Na series	
1	.9526 g.	1	.0420 g.	1	1.0140
2	.9284	2	.0520	2	.9054
3	.9636	3	.0498	3	.8824
4	.8810	4	.0740	4	.9512
5	.9828	5	.0616	5	.9124
6	.9490	6	.0948		
7	.9336	7	.0508		
Average	.9416 g.	Average	.0607 g.	Average	.9331

The ratio of the weight of the Mg cultures to the weight of the Na cultures is 15.5. Evidently sodium can not replace magnesium, and the meagre growth obtained on the Na cultures may perhaps be due to traces of Mg in the solutions used, or as BENECKE has pointed out, to a dissolving out of the Mg constituent of the Jena glass, by the nutrient solution being rendered acid by the presence of the fungus. The sodium, although evidently not used by the fungus, produced no marked deleterious effect when present in the nutrient solution. Three cultures of each of the Mg and the Na series were not filtered, but allowed to continue in order to ascertain whether or not further development would be possible on the Na series. Even after a period of two months there was no discernible increase in the amount of mycelium and no spores were formed.

Experiment II.

In this experiment, an equi-molecular quantity of Beryllium was substituted for the Mg of the standard nutrient solution. Ammonium sulphat was used as a source of sulphat. Twenty-five cc. of this salt were added to 1 liter of solution. This amount of $(NH_4)_2SO_4$ was not based upon any calculations, and it was later discovered that the estimate was far too high, as 10.76 cc. gave an equivalent weight of sulphat to that of the standard nutrient solution. One hundred cc. of NH_4NO_3 were added in place of the 125 cc. of the standard nutrient solution. This amount was likewise estimated. The nutrient solution was as follows: 1000 cc. of solution contained:-

100 cc. NH_4NO_3
25 cc. $(NH_4)_2SO_4$
50 cc. KH_2PO_4
6.5 cc. $BeCl_2$
5 cc. M/100 $FeCl_3$
50 gr. sucrose

In making up the solution, it was found that a precipitate was formed, when the beryllium chloride was added, however, it was decided to continue the experiment, care being taken to distribute the precipitate equally among the culture flasks.

A parallel series of cultures, containing the standard nutrient solution, was used as a control. All cultures were allowed to continue for 22 days at a temperature of 20 - 22°C.

The Be-series showed no signs of growth, germination of the spores not even occurring. The standard nutrient cultures produced a heavy growth and were apparently normal in all respects. No dry weight determinations were made.

Experiment III.

Preliminary to performing this experiment, the cause of the precipitate by beryllium chlorid in the preceding experiment was determined. Investigation showed that the precipitate was due to a reaction between beryllium chlorid and the potassium phosphat. An insoluble phosphat of beryllium was evidently formed. This led to the possibility that in the beryllium series of the preceding experiment, the failure of the fungus to grow might have been due to a lack of available phosphat rather than to the failure of beryllium to replace magnesium. Attempts to substitute other phosphates for that of potassium, resulted in the same precipitate being formed. It was found that by supplying the potassium as KCl and the phosphorus as phosphoric acid no precipitation occurred, when beryllium was added to the nutrient solution. The amounts of KCl and H_3PO_4 to be added were calculated so that the nutrient solution received amounts of K and P equal in weight to those of these elements in the standard nutrient solution. The addition of the phosphoric acid rendered the nutrient solution quite acid, but as soon as KOH or NaOH was added to neutralize the solution, an immediate precipitation occurred. It was, therefore, decided to attempt to grow the

fungus on the acid solution. In all experiments where $MgSO_4$ was removed from the nutrient solution, the sulphat was added in the form of $(NH_4)_2SO_4$. The amount of this salt added was such as to give a weight of sulphat equal to that in the standard nutrient solution. To solutions to which $(NH_4)_2SO_4$ was added, enough NH_4NO_3 was added so that the total nitrogen content of the solutions was equal in weight to that of the standard nutrient solution.

In Experiment III, magnesium was replaced by an equivalent weight of beryllium; the latter being supplied as beryllium chlorid. The nutrient solution contained in 1000 cc. was as follows:

118.5 cc. NH_4NO_3
10.76 cc. $(NH_4)_2SO_4$
36 cc. H_3PO_4
27.4 cc. KCl
5 cc. M/100 $FeCl_3$
50 g. sucrose
17.5 cc. $BeCl_2$

A parallel series, containing the standard nutrient solution, was used for a control. The cultures were placed in a dark room at a temperature of 20 - 22°C for twenty-one days. During that time the growth on the standard nutrient cultures was in all respects normal. Germination of the spores on the Be-series did not even occur. In three cultures of this series, allowed to run for two months, there developed a small amount of mycelium. In two of these cultures, which were in the dark room, the mycelium was

practically all submerged, there appearing but a single small tuft on the surface of one of the cultures. In the third culture, placed in the incubator at 30°C, there was considerably more mycelial development than in the other two. A fair portion of the mycelium was also on the surface. None of the mycelium in any of the cultures showed any signs of spore formation. The cause for this mycelial growth in these three cultures may perhaps be attributed to the dissolving of the Mg-constituent of the glass flasks by the acid nutrient solution. BENECKE has shown that Jena glass is less resistant at higher than at lower temperatures which may explain the greater growth on the culture at 30°C.

The failure of growth to appear in the Be-series may have been due to, (1) the inability of Be to replace Mg, (2) the acidity of the nutrient solution, and (3) the fact that the Be may have been present in too great a concentration.

Experiment IV.

In order to determine whether the failure of the fungus to develop in the Be-series of the preceding experiment was due to the acidity of the nutrient solution, the effect of replacing the KH_2PO_4 of the standard nutrient solution by KCl and H_3PO_4 was studied. The nutrient solution contained per 1000 cc.

125 cc. NH_4NO_3
36 cc. H_3PO_4
27.4 cc. KCl
20 cc. $MgSO_4$
5 cc. M/100 $FeCl_3$
50 gr. sucrose

A parallel series for a control contained standard nutrient solution. All flasks were allowed to remain for 11 days at a temperature of 20 - 22°C. Growth was at first somewhat faster on the cultures containing the standard nutrient solution; however, at the time of filtering there was no appreciable difference, either in growth or in sporulation between the cultures of the two series. The dry weights are as follows:

Standard nutrient solution		H_3PO_4 + KCl series	
1	.6934 gr.	1	.9496 gr.
2	1.0512	2	.8320
		3	.8880
		4	.9262
		5	1.0390
Average	.8723	Average	.9270

Since there were but two cultures containing standard nutrient solutions, and as the dry weights of these two cultures varied considerably, it would not be safe to draw any sharp conclusions. It is nevertheless very evident that the failure of the fungus to grow in the Be-series of Experiment III can hardly be attributed to the acidity of

the nutrient solution.

Experiment V.

This experiment was performed with the object of determining the effect of reducing the amount of K_2HPO_4 used in the nutrient solution of the preceding experiments. Besides the nutrient solution used in Experiment II, there were used solutions in which the amount of K_2HPO_4 was reduced to 2/3 and to 1/3 of the original amount. No other changes were made in the content of the nutrient solutions. The three solutions used contained per [illegible] cc. of K_2HPO_4 respectively. All the solutions were placed at a temperature of [illegible] - 22° and [illegible] days. [illegible] the smaller amount of K_2HPO_4. [illegible] the least amount of K_2HPO_4 was [illegible]. [illegible]

[illegible]

36 cc. H_3PO_4 per 1000 cc.		24 cc. H_3PO_4 per 1000 cc.		12 cc. H_3PO_4 per 1000 cc.	
1	1.0638 g.	1	1.1176 g.	1	.8170 g.
2	1.1248	2	.5788	2	.9534
3	.8218	3	.7804	3	.8912
4	1.0676	4	1.1502	4	1.1668
Average	1.0195	Average	.9068	Average	.9571

Decreasing the amount of H_3PO_4 within the limits used in this experiment did not lead to better results, hence it was decided to retain the original solutions for the use in all experiments where Beryllium was to be substituted for magnesium.

Experiment VI.

Having eliminated the possibility that the H_3PO_4 prevented the growth of Aspergillus in cultures containing Be but no Mg, it was decided to attempt the replacement of Mg by using smaller quantities of Beryllium. Three nutrient solutions were used containing per 1000 cc.

A	B	C
118.5 cc. NH_4NO_3	118.5 cc. NH_4NO_3	Same as A, + 20 cc. $MgSO_4$
10.76 cc. $(NH_4)_2SO_4$	10.76 cc. $(NH_4)_2SO_4$	
36 cc. H_3PO_4	50 cc. KH_2PO_4	
27.4.cc. KCl	5 cc. M/100 $FeCl_3$	D
5 cc. M/100 $FeCl_3$	4 cc. $BeCl_2$	Standard nutrient solution
4 cc. $BeCl_2$	20 cc. $MgSO_4$	
50 gr. sucrose	50 gr. sucrose	

All cultures were allowed to continue for eleven days at a temperature of 30°C. Solution B was made up accidentally, the KH_2PO_4 being added when it was intended to add KCl and H_3PO_4. After realizing the mistake, it was decided to retain the solution and note the growth which might be produced on it.

The cultures of series A showed a very feeble mycelial growth. In most of the flasks there were 2 - 4 masses of submerged mycelium, each about one centimeter in diameter. On two of the cultures there was a single tuft of surface mycelium of similar size and on these were borne solitary dwarfed and evidently immature sporangiophores.

The cultures of series B showed a heavy growth and very heavy sporulation, at least equal if not greater than that of the cultures of series D. No toxic effect, due to the presence of beryllium was evident, but judging from external appearances one might well believe that the beryllium had exerted a stimulatory influence.

A quite different effect was to be noted on the cultures of series C. These showed a fairly good mycelial growth, yet the sporulation was not as heavy, and on the whole a toxicity was very evident. The nutrient solution presented a very yellow appearance.

The development of the fungus on the standard nutrient solution was typical in every way.

The dry weights were determined to be as follows:

- Mg + Be		+ Mg + Be		+ Mg + Be		+ Mg - Be	
Series A		Series B		Series C		Series D	
1	.0098g	1	.9830g.	1	1.0010 g.	1	1.0360
2	.0260	2	1.0046	2	.9646	2	1.0564
3	.0214	3	1.0164	3	.9992	3	1.0400
4	.0314	4	1.0900	4	.9750	4	.9696
5	.0124	5	.9624	5	1.0124		
Average	.0202	Average	1.0113	Average	.9904	Average	1.0255

The dry weights in the above table are somewhat surprising. It is interesting to note that the average for series C, in which a toxic effect seemed apparent, is only slightly less than that of the series B, in which a stimulatory effect seemed to be present. Both these series possessed an average dry weight nearly equal to that on the standard nutrient solution. In fact, the differences are too small to warrant any conclusions, other than in the presence of magnesium, this concentration of beryllium had little or no injurious effect. The average dry weight of the Series A was very low, and might easily have resulted from impurities in the solution, or as BENECKE has mentioned, from traces of magnesium present in the spores used for inoculating the cultures.

Experiment VII.

In order to ascertain whether the beryllium had been

responsible for the growth on the cultures of Series A in the preceding experiment, it was decided to attempt the growth of the fungus on a solution to which neither beryllium nor magnesium was added. For a nutrient solution, that of Series A of the preceding experiment was used, with the exception that the beryllium chlorid was omitted. The cultures were grown for eleven days at 30°C just as in the preceding experiment. The mycelial growth was very slight, appearing as scattered tufts on the surface, or still more as submerged masses of one-half to one centimeter in diameter. The surface mycelium was very white and bore only an occasional solitary sporangiophore, which did not appear to reach maturity.

The dry weights were:-

1	2	3	4	5	Average
.1596 g.	.0762	.0754	.0782	.1034	.0985

Although very small, the average dry weight was nearly five times that of series A of the preceding experiment. From this it might be concluded that the beryllium did not tend to increase the mycelial development in the previous experiment but instead it exerted a depressant influence.

Experiment VIII.

Since no marked decrease in dry weight had been induced by the presence of Be in series B and C in Experiment VI, it was decided to study the effect of still smaller amounts of

beryllium. The nutrient solutions were the same as those of series A and C of Experiment VI with the exception that just one-half the amount of beryllium was added to each. Two cc. of $BeCl_2$ were then present in these solutions. All cultures were grown for twelve days in the incubator at 30°C.

The cultures containing beryllium but no magnesium presented an appearance very similar to those of Series A of Experiment VI. The mycelial development was slight, and had the usual tuft-like growth mainly submerged but a few tufts on the surface. A few partially developed sporanges were also to be found growing on the surface mycelium.

The cultures with both beryllium and magnesium had the same toxic appearance as did those of Series C of Experiment VI. There was a good mycelial growth but a decrease in the amount of sporulation. As this experiment was performed under exactly the same cultural conditions as Experiment VI, the dry weights of the standard nutrient culture of that experiment may serve as a basis of comparison here.

The dry weights were as follows:-

+ Mg - Be

$- MgSO_4 + BeCl_2$		+ Mg + Be		Standard nutrient	
1	.0208 g.	1	.8990 g.	1	1.0360
2	.0078	2	.9182	2	1.0564
3	.0080	3	.8538	3	1.0400
4	.0082	4	.8822	4	.9696
5	.0150	5	.8658	5	
Average	.0119 g.	Average	.8838 g.	Average	1.0255

The average dry weights for the Be and Be + Mg cultures are lower than those of similar cultures in Experiment VI. The only conclusion which may be drawn is that the addition of smaller amounts of beryllium produced no beneficial effect.

Experiment IX.

In this experiment it was decided to repeat the work of Experiment VI and in addition to use nutrient solutions which contained only one-tenth of this amount of beryllium chlorid. Five solutions were used: (A) containing 4 cc. of Beryllium chlorid per 1000 cc., (B) containing the same amount of $BeCl_2$ as in A, but also the standard amount of $MgSO_4$, (C) the same as A except only .4 cc. of $BeCl_2$ were added, (D) the same as (B) except only .4 cc of $BeCl_2$ were present, (E) the standard nutrient solution. All cultures were allowed to continue for 12 days at a temperature of 21 - 23°C.

No growth, not even spore germination, occurred on the

cultures of Series A. The cultures of Series B developed a good mycelial growth and a heavy sporulation. The mycelial growth was evidently not as great as that on the standafd nutrient solution. However, there was little difference in the degree of sporulation. In Series C, a very slight flaky growth of mycelium appeared in all the cultures. The growth was in part superficial and in part submerged. No sign of sporulation was evident. Between the growth and sporulation in Series D and E there was no marked difference, except possibly the mycelial growth in D appeared somewhat heavier than on the standard nutrient solution.

The dry weights resulted as follows:

Series A		Series B		Series C		Series D		Series A	
1	0 g.	1	.5942 g.	1	.0440 g.	1	1.0852g	1	1.2322 g.
2	0	2	.9264	2		2	1.0788	2	1.1278
3	0	3	1.1930	3		3	.8312	3	ml.0854
4	0	4	1.1126	4		4	.9062	4	1.0474
Aver.	0	Aver.	.9566	Aver.	.0110	Aver.	.9754	Aver.	1.1232

The results show that the presence of beryllium did not increase the dry weights of the fungus, but rather caused a decrease. A greater decrease was produced in the cultures containing the greater amount of beryllium chlorid. However, here the injurious effects of the beryllium were not great. Again as in preceding experiments beryllium failed to cause

growth in cultures to which no magnesium was added. It is very evident that beryllium is unable to assume the functions of magnesium in the nutrition of Aspergillus niger, consequently further attempts at substitution were not made.

Experiment X.

After being unable to replace magnesium by the closely related element, beryllium, it was decided to study the effect of varying the amount of $MgSO_4$ in the nutrient solution. The standard nutrient solution was used as a basis for comparison. In addition to this species four others were made, (1) containing 1/4 the normal amount of $MgSO_4$, (2) 1/2, (3) twice the normal amount, (4) three times the normal amount. The five series then contained per 1000 cc., 5, 10, 20, 40, and 60 cc. of $MgSO_4$ respectively. All cultures were placed at a temperature of 21 - 23°C and allowed to continue for 14 days. Growth in the five series bore the same relationship as did the $MgSO_4$ content, being greatest on those cultures containing the largest amount of $MgSO_4$. The degree of sporulation considered from the relative amount of mycelial growth, was about equal in all of the series.

Dry weight determinations were made with the following results.

1/4 normal	1/2 normal	normal	2 x normal	3 x normal
1: .4510 g	1: .8590 g.	1: 1.2558 g	1: 1.0656 g.	1 : 1.0946 g
2: .6614	2: .9610	2: .8808	2: .9120	2: 1.1236
3; 1.0056	3: .8670	3: 1.0566	3: 1.1920	3 : 1.0944
4: .9500	4: .9490	4: .5910	4: .9656	4 : .9976
Aver.7670	Aver..9090	Aver.9460	Aver.1.0338	Aver.1.0775

The dry weights show the greatest average in the series containing the largest amount of $MgSO_4$. Although the dry weights bear to each other the same general relationship as the $MgSO_4$ content of the different series, they are not directly proportional to it. Increasing the amount of $MgSO_4$ to twice the normal amount did not double the dry weight of the fungus produced. Within the limits of this experiment the dry weights increased much more slowly than did the $MgSO_4$ content. The optimum $MgSO_4$ content was evidently not reached in this experiment as the dry weights still showed an increase in the series containing the highest $MgSO_4$ content.

V. DISCUSSION OF RESULTS

The above described experiments give results similar to those of the great majority of investigators. All attempts to grow Aspergillus without an addition of magnesium to the nutrient solution met with failure. In all cases except one, germination of the spores did occur in solutions to which no magnesium was added; however, the growth which followed

was very slight and spores were never formed. This agrees with the results of BENECKE, RAULIN, GÜNTHER and other investigators. COUPIN and FRIEDEL, however, report that Sterigmatocystis versicolor forms spores when no magnesium is added to RAULIN'S nutrient solution. Most workers are inclined to believe that growth, which occurs when no magnesium is added to the nutrient solution, must be due to traces of magnesium (1) which are not removed from the nutrient solution; (2) which dissolve from the cultural flasks, or (3) which are in the spores when sown. COUPIN and FRIEDEL seem inclined to believe that their results were due to the nature of the organism with which they worked, rather than to the presence of magnesium in the nutrient solution.

In one series of my experiments, the spores of Aspergillus failed to germinate in a nutrient solution to which magnesium was not added. This may have been due to the total absence of magnesium, or to the toxic effect of beryllium which was also present in the nutrient solution. However, Aspergillus spores were able to germinate in nutrient solutions which contained a much higher beryllium content than did that of the cultures in question. MOLISCH reports that when ammonium tartrat was used as a source of carbon and no magnesium was added to the nutrient solution, spores of Penicillium and Aspergillus failed to germinate. This result he ascribes to the total absence of magnesium

in the nutrient solution.

As BENECKE, MOLISCH, GÜNTHER and JAVILLIER have stated in their work with Penicillium and Aspergillus, so in my experiments with Aspergillus niger, beryllium was found unsuitable for replacing magnesium. NOESSKE received similar results in his experiments with Bac. pyocyaneus.

Beryllium was not found to be a very strong poison. Aspergillus niger was able to grow to a very slight extent in solutions containing .175% $BeCl_2$. BENECKE states that beryllium is not toxic to Aspergillus and Penicillium unless it be concentrated to a considerable degree. GÜNTHER found beryllium to be the least toxic of any of the alkaline earths of the zinc group. He found that up to 0.2%, beryllium could be tolerated in the nutrient solution. JAVILLIER using amounts of Be equivalent in weight or molecular quantity to the magnesium in RAULIN'S solution, obtained no growth of Aspergillus niger. His experiments continued but for four days and hence are subject to some criticism.

In no case did the addition of beryllium to a standard nutrient solution produce a stimulatory effect. A depressant effect was always to be noted as is indicated by the dry weight determinations.

BENECKE has shown that a $MgSO_4$ content of .25% produced the maximum growth of Aspergillus. By increasing the $MgSO_4$ content to as high as three times that of the standard nutrient

solution, my experiments always showed an increase in the dry weight of the fungus. Decreasing the amount of $MgSO_4$ caused a decrease in the dry weights. In my experiments, as in the work of other investigators, the dry weight is not directly proportional to the $MgSO_4$ content of the nutrient solution.

VI. SUMMARY

1. Magnesium was found to be essential for the complete nutrition of _Aspergillus niger_.

2. Very small amounts of magnesium are sufficient for vegetative growth; however, somewhat larger amounts are necessary for spore formation.

3. Replacement of Mg by Be was found to be impossible.

4. Beryllium was found to possess no value as a mineral nutrient, even when added to solutions containing magnesium.

5. _Aspergillus niger_ was able to grow to a slight extent in nutrient solutions containing an amount of Be equivalent in weight to the Mg contained in the standard nutrient solution. This inhibition of growth shows a certain degree of toxicity.

6. Beryllium never produced a stimulatory effect on the growth of Aspergillus, but rather exerted a depressing influence.

7. Increasing the amount of the $MgSO_4$ at least three times that contained in the standard nutrient solution always produced a certain increase in the dry weight of the fungus.

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